Project Report: Iron Oxidation – Shaping the Past and Present Environments

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Project Progress

The principal objective of our project is to develop a better understanding of the fundamental biology of microbial iron (Fe) oxidation at neutral pH. Though the capacity for Fe oxidation is likely an ancient metabolic pathway, it is to date the least well understood among the major microbial metabolisms. We secondarily wish to explore the potential for Fe oxidation among eukaryotes, using the acid mine drainage system “Rio Tinto” and the eukaryotic culture collection as a model system.

Toward our first objective, we have identified an ideal model bacterium that we have focused most of our efforts on in the past year. Our deep–sea Fe–oxidizing culture collection consists entirely of obligate Fe–oxidizing autotrophs. Many of these are gamma Proteobacteria that are closely related to the common marine heterotrophs *Marinobacter* and *Halomonas* . In testing available cultures of these bacteria, we found that many of them are facultative autotrophs and/or mixotrophs that are capable of oxidizing Fe. We have developed one of them, *Marinobacter aquaeoloi* , as a model bacterium for studying Fe oxidation, because of its advantageous growth characteristics. Using SDS–PAGE gel electrophoresis of cell extracts we have determined that a 35 kDa protein is up regulated with increasing Fe(II) content in medium. We have also developed a plate–based screening assay for the detection of Fe oxidation. This plate–based method is being used to develop a genetic system in *M. aquaeoloi,* which we have determined to be competent, and are in the process of optimizing conditions for transposon mutagenesis. This plate screen will also be used for our second objective with eukarya. Finally, we are working towards genome sequencing of *M. Aquaeoloi* and toward this end have extracted high–molecular–weight DNA and sized the genome, which is 2.555 –2.595 kb. Large insert libraries have also been made (Fosmid).

Highlights

- Identified “model” Fe–oxidizing bacterium: *M. aquaeoloi*.
- Developed a plate–based screening method for *M. aquaeoloi*.
- Determined that Fe oxidation is upregulated in *M. aquaeoloi* in the presence of Fe(II) and a 35 kDa protein can be detected.

- *M. aquaeoloi* is competent and a genetic system is being developed.
- A fosmid library has been constructed with *M. aquaeoloi* DNA.
- The genome of M. aquaeoloi has been sized at 2.555–2.595 kb.

Roadmap Objectives

- ***Objective No. 4.1:*** Earth's early biosphere

Cross Team Collaborations

I collaborate extensively with Dave Emerson on the topic of neutraphilic iron oxidizing bacteria. We have a collaborative field project at Loihi seamount. The methods that we are developing using cultures, specifically the development large insert libraries and application of our plate screening mehod will be used in the collaborative project with Emerson with environmental template material.